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Training software for chiral separations in capillary electrophoresis

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Abstract

A previously published steady-state simulation program for CE was extended with a sub-menu for chiral interaction. The interaction was modelled with a hypothetical (neutral) selector with properties similar to cyclodextrins. A three-type chiral interaction model was implemented in such a way that it was valid for both anionic and cationic analytes. User-defined chiral variables in the model were: the concentration of this chiral selector, the complex formation constants (K) of both non-ionic and ionic analyte species, the relative K differences between the two optical isomers (also for both non-ionic and ionic species) and the mobility of the analyte–selector complex, relative to that of the fully dissociated free analyte. In addition the following non-chiral variables are added: the dependence of the bulk viscosity on the chiral selector concentration and the dependence of the mobilities on the viscosity. Chiral components in the data base are simulated as mixtures of optical isomers with variable enantiomeric concentration ratios. These chiral parameters, together with the original features of the program, provide a very flexible training tool for chiral separations in capillary electrophoresis. For example, determination of the optimum chiral selector concentration can be done in seconds, using the simulation software. Although the software is intended mainly for training purposes, in cases where sufficient details of existing chiral selector–analyte combinations are available, the program is also suitable for method development of chiral separations in CE.

The performance of the program is illustrated with simulations, using chiral parameters obtained from literature (ibuprofen, fenoprofen, homatropine) and from our own experiments (*m*-fenfluramine). Simulation results were compared with experimental electropherograms.

Keywords: Computer simulation; Enantiomer separation; Electrophoretic mobility; Viscosity; Buffer composition; Fenfluramine; Fenoprofen; Ibuprofen; Homatropine

1. Introduction

For training purposes, fast simulation programs based on steady-state models were found to be a useful addition to real laboratory experiments [1]. This was already obvious for techniques such as gas chromatography [2] and micellar electrokinetic chromatography [3], where elementary equilibrium thermodynamics can be readily used to model the temperature dependent partition between mobile and (pseudo) stationary phases.

In capillary electrophoresis however, the fundamental basis for separation behavior is the concept of mobility, which together with basic acid–base equilibria, can be used to model the separation pattern in detail. A steady–state simulator based on such a model is now available for training purposes [4,5]. Although in that simulator, temperature and ionic strength effects are modelled, using emperical relations, complications such as complex formation and stereoselectivity were not taken into account. The use of electrophoretic techniques for separation of optical isomers has by now gained widespread attention. Recent reviews on the subject [6,7], with

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hundreds of references, list many applications of the use of cyclodextrins in capillary zone electrophoresis, micellar electrokinetic chromatography, electrokinetic chromatography, as well as some other chiral selectors. In spite of the large number of references on chiral applications now available, quantitative data on modelling the chiral interaction are not easily obtained. A three-type interaction model for chiral interaction of acidic [8] and basic compounds [9] was clearly outlined by Rawjee et al. Both ionic and non-ionic interaction of either of the two optical isomers were quantified with corresponding complex formation constants. If, in addition the effective mobility of the free analyte and of the analyte–selector complex are known, a 6 parameter model is obtained that was confirmed to agree well with experimental results of selectivity optimization [8,9]. The present study was carried out for the benifit of a EU-sponsored TEMPUS Joint European Project 'New Curriculum in Chiral Chemistry', resulting in a curriculum given at the Comenius University in Bratislava during the academic years 1995/1996 and 1996/1997. For the subject of capillary electrophoresis, simulation experiments with the previously published HPCESIM simulation program [3,4] were planned as the introduction[1]. For the introduction to the chiral part of the practical course, this simulation program was extended with a submenu for chiral interaction, based on the above mentioned chiral parameters.

2. Experimental

2.1. Simulation and experimental conditions

The simulation software was programmed and compiled in PowerBasic version 3.0 (PowerBasic, Carmel, CA, USA). The computer hardware was an IBM-compatible PC with a Pentium 120 processor, 8 MB RAM, 1 GB Harddisk and VGA monitor. For running the software, a 386 type PC with 33 MHz clock speed is a minimum requirement in order to keep calculation times to within a fraction of a

second. This enables the 'instant' response necessary for use of short-cut keys.

Simulated and real experimental equipment conditions correspond to a usual commercial CE instrument (P/ACE, Beckman, Fullerton, CA, USA). Equipment conditions were: capillary 568 cm (500 mm to detection)×50 µm I.D., voltage 10 or 20 kV. Injection 0.5 psi (ca. 3300 Pa) during 5 s. The instrument was thermostated at 25°C. The ionic strength of the background electrolyte (BGE) was 10 m*M*. BGEs were prepared with analytical grade chemicals from the usual sources (Merck, Darmstadt, Germany or Sigma, St. Louis, MO, USA). Heptakis-2,3,6-tri-O-methyl-β-cyclodextrin (TRIME-β) was from Cyclolab (Budapest, Hungary). *meta*-Fenfluramine[N-ethyl-α-methyl-(trifluoromethyl)phenethylamine hydrochloride] as Ponderal™ (racemate) and as Isomeride™ were from Servier Technologie (Paris, France).

3. Results and discussion

3.1. Chiral sub-menu

The following user-defined variables were provided as model input: the concentration of the chiral selector, the complex formation constants of both non-ionic (K_1) and ionic analyte species (K_2), the relative K differences $(\delta K_1$ and $\delta K_2)$ between the two optical isomers (also for both non-ionic and ionic species) and the mobility of the analyte–selector complex, relative to that of the fully dissociated free analyte. Chiral components in the data base are simulated as mixtures of optical isomers with variable enantiomeric concentration ratios.

When adding chiral selectors such as cyclodextrins to the BGE, the bulk viscosity often increases and as a result, the ohmic resistance of the capillary adjusts according to the decreased mobilities. These effects can be of the order of 10%. The bulk viscosity was already included in the program as an independent variable. Previous experiments have shown that for β-CD polymer, the viscosity increases with the β-CD concentration according to a quadratic relation:

$$\eta/\eta_0 = 1 + a_1 C_s + a_2 C_s^2 \tag{1}$$

in which η_0 is bulk viscosity without chiral selector and C_s the analytical concentration of the chiral selector. The coefficients a_1 and a_2 are now included as program parameters. The mobility–viscosity relation in non-sieving media was estimated by monitoring the current at constant voltage as a function of the BGE viscosity. It was observed that the BGE mobilities μ decreased linearly with increasing viscosity according to the relation:

$$\mu_0^*/\mu_0 = 1 + a_3\eta/\eta_0 \qquad (2)$$

in which μ_0^* is the mobility of the fully charged ion in water and a_3 is a constant that surprisingly was found to be 0.78 for the β-CD polymer mentioned [10]. In other cases, values of a_3 close to zero were observed [11]. For this reason, the constant a_3 was also included as a parameter.

Table 1 summarizes the additional chiral and non-chiral parameters with their allowed range of values. The interaction type listed refers to the definitions introduced by Rawjee et al. [8,9]. All these chiral parameters can be loaded and saved as buffer files, together with the other buffer parameters (pH, composition and ionic strength).

3.2. Calculation of migration behavior

Migration behavior in chiral systems is rather more complicated than in non-chiral systems. For example, an acidic analyte can be present in either of four species: as a charged or as a non-charged acid (equilibrium subject to K_a), as a non-charged acid complexed with the chiral selector (subject to K_1) and as a charged acid complexed with the chiral

selector (subject to K_2). Only two of these species migrate. The effective mobility of chiral components in the presence of chiral selector μ_{eff} is calculated from the mobility of the fully charged ion μ_0 according (for monovalent anions) to the following equation [8]:

$$\mu_{eff} = \mu_0 \cdot \frac{1 + R_M K_2 C_s}{1 + K_2 C_s + (1 + K_1 C_s)[H_3O^+]/K_a} \qquad (3)$$

in which R_M is the mobility of the analyte–selector complex divided by the mobility of the fully ionized free analyte, $[H_3O^+]$ the hydrogen ion concentration and K_a the acid dissociation constant of the analyte. The values of μ_0, R_M and K_a are the same for both isomers, whereas the values of K_1 and K_2 may differ. For cations, Eq. (3) transforms into:

$$\mu_{eff} = \mu_0 \cdot \frac{1 + R_M K_2 C_s}{1 + K_2 C_s + (1 + K_1 C_s)[OH^-]/K_b} \qquad (4)$$

in which K_b is the base dissociation constant. For multivalent ions, the denominators of Eq. (3) and Eq. (4) are accordingly adjusted. It should be emphasized that Eqs. (3) and (4) are valid only in cases where the analyte concentration is much smaller than C_s. Consequently, the model does not describe sample overload.

In the software, two kinds of sample components are distinguished: those with potential chiral properties (the names are stored in an auxillary ASCII-type file) and those without such properties. The former are all treated alike, in that they are supposed to interact with the hypothetical chiral selector in

Table 1
Chiral parameters in the HPCESIM simulation program

Variable	Dimension	Range from	To
Interaction type	–	0 (none-chiral)	3 (ionic and non-ionic)
Selector concentration mmol/l		0	200
K_1 (non-ionic interaction)	–	0	2000
δK_1 (+ vs. −)	%	−25	+25
K_2 (ionic interaction)	–	0	2000
δK_2 (+ vs. −)	%	−25	+25
R_M (relative mobility)	%	0	99
Purity (% +)	%	0.1	99.9
a_1 in Eq. (1)	–	0	5
a_2 in Eq. (1)	–	0	100
a_3 in Eq. (2)	–	0	2

exactly the same manner (all chiral complexation constants are the same for all chiral analytes).

The non-chiral analytes also interact with the selector, again with the same K_1 and K_2 values, but the δK_1 and δK_2 values are set to zero. Although not set as a separate goal for the current version of the program, in this way some form of (non-chiral) complexation can be modelled as well, with the limitation of a non-charged complex forming agent.

As long as the chiral selector concentration is zero, program performance remains exactly what it used to be. Only with non-zero selector concentrations is additional information is available: selectivity and resolution now refer to the separation of the respective optical isomers, resulting in zero values for non-chiral analytes. Other tabulated information on migration and dispersion always refers to the last migrating optical isomer.

3.3. Visualization of results

As an example, a number of cations with potential chiral properties were simulated with both K_1 and K_2 set to 100. The δK_1 value is zero, δK_2 is set to 5%, the relative mobility to 10% and the optical purity to 40%. This is a type 2 interaction where only the ionic form of the analytes shows chiral discrimination by the selector. In Fig. 1a, the analysis under non-chiral conditions were shown, where the sepa-
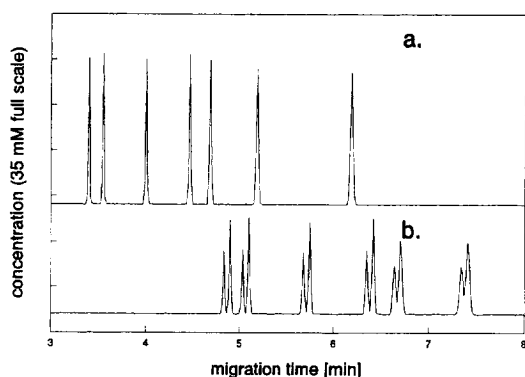
ration is optimized by a BGE pH in the range of the pK values of the analytes, so that they were separated according to their pK value. Fig. 1b simulates a chiral selector concentration of 10 mmol/l.

3.4. Selectivity vs. resolution

As seen from Fig. 1, chiral resolution is complete for the first few analytes. The last migrating components show less resolution because of two reasons. The degree of ionization of these is less, so that with a type 2 interaction less selectivity can be expected under these conditions. In addition to this difference in selectivity, resolution is also less because at increased migration times, increased dispersion due to diffusion is expected. From the point of selectivity, the model proposed in the literature [8,9] appears to form a complete description of interaction phenomena. The resulting equations for selectivity are independent of all μ_0 values, under the assumption that the charged analyte–selector complex mobility has no chiral distinction. Whether or not racemic mixtures can be separated, also depends on additional peak-broadening contributions from injection volume, concentration overload, diffusion, radial temperature gradient, detector cell length and time constant. These additional effects are included in the present simulations as well.

3.5. Comparison with literature results

Unfortunately, most publications on chiral separations lack quantitative information on the complex formation constants that can be used to model the analyte–solute interactions. In their publications describing the previously mentioned type 1–3 interaction mechanism, Rawjee et al. [8,9] give a number of examples for anionic and cationic sample components. Results of the optimization strategy were presented as selectivities vs. pH and selector concentration plots. It will be shown that in addition the present software is capable of calculating not only these selectivities, but also the resolution, often a more practical optimization criterion (as long as analysis time is considered as well). Some examples will now be outlined.

Mobilities and complexation constants for β-cyclodextrin interaction with fenoprofen and ibu-

Fig. 1. Simulation of electrophoretic separation (in the elution order) of procaine, β-eucaine, cocaine, diocaine, psicaineleu, tetracaine and bupivacaine respectively without (a) and with 10 mM chiral selector (b). The buffer is a 10 mM Tris–borate at pH 8.0. Further conditions as described in the Section 3.3.

profen were determined [8]. Based on these values, a simulation was carried out at pH 4.5, in a 200 m*M* 2-(N-morpholino)ethanesulfonic acid (MES) buffer with 15 m*M* β-CD, using the equipment conditions as mentioned in the original article. In both instances there is a type 1 interaction with the β-CD, meaning stereoselective complexation of the non-ionic form of the analytes. Chiral selectivity surfaces as a function of pH and CD concentration [8] show monotonous increase of selectivity with decreasing pH and increasing CD content. Both components were simulated separately because the chiral conditions in the program are always the same for all chiral analytes present in the sample. The results (Fig. 2) agree reasonably well with the experimental electropherogram (Fig. 3).

In another example, the chiral separation of the cation homatropine [9], chiral interaction is of type 3, meaning that both the ionic and non-ionic form of the analyte exhibit stereoselective interaction with the β-CD, although to a different extent. In this example, Rawjee et al. [9] found for K_1 1328, δK_1 3.4% and K_2 95.9, δK_2 8.4%. In other words, the non-ionic form shows stronger but less stereoselective interaction, the ionic form shows weaker but more stereoselective interaction. Although in the practical concentration range of β-CD (0–15 m*M*) the migration sequence remains the same and there is a distinct selectivity optimum, at higher hypothetical concentrations there is a selectivity reversal. Fig. 4
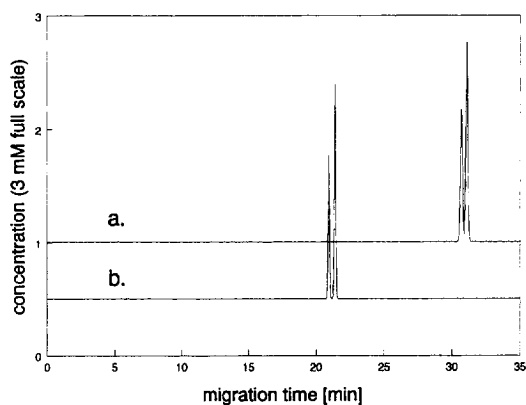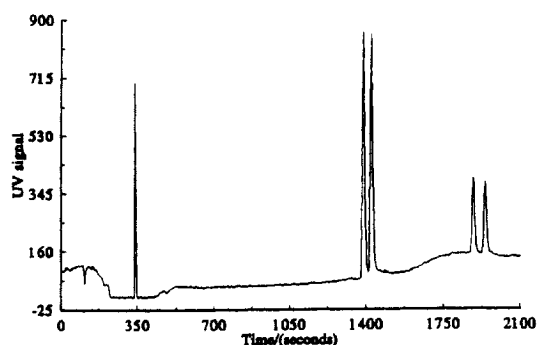

Fig. 3. Capillary electrophoretic separation of ibuprofen and fenoprofen respectively, conditions as in Fig. 2. Reprinted from Ref. [8] with permission.

illustrates analysis at three different chiral selector concentrations. Obviously, if possible, use should be made of K_2 because of shorter analysis times.

3.6. Comparison with experimental results

As another example, the chiral separation behavior of optical isomers of meta-fenfluramine was investigated with CE using TRIME-β as chiral selector.

Fenfluramine is a base with listed pK of 9.1 [12] that is mainly administered as an anti-obesity drug. In the Netherlands, it is commercially available both as a racemic mixture (Ponderal) and as an optically pure *d*-isomer (Isomeride). The latter is the biologically active component. The optical purity of
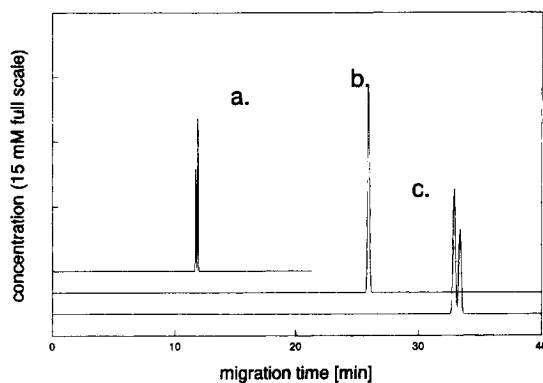

Fig. 2. Simulation of electrophoretic separation of ibuprofen (a) and fenoprofen (b) in 200 m*M* MES, pH 4.5, chiral selector concentration 15 m*M*. Other conditions in Ref. [8].

Fig. 4. Simulation of electrophoretic separation of homatropine at 5, 25 and 50 m*M* hypothetical chiral selector with model parameters and experimental conditions as in Ref. [9]. The buffer was 10 m*M* phosphoric acid–LiOH to pH 9.0.

Isomeride was investigated by Porrà et al. [13], using capillary electrophoresis with TRIME-β as chiral selector. It amounted to ca. 98%. This was confirmed by our analyses.

First, the pK and mobility of m-fenfluramine under experimental CE conditions were determined: 25°C and a ionic strength of 10 mM, slightly different from those reported (15°C and ca. 100 mM) [13]. Different BGE's were prepared by adding boric acid to a sodium hydroxide solution with a final concentration of 10 mM. Mobilities were determined by injection of mesityloxide as neutral marker, according to the recently published dual-marker method by Williams et al. [14]. The inverse values of the resulting effective mobilities were plotted vs. the hydroxide ion concentration in the range pH 8.6 to 9.4. The following relation is valid for cations:

$$\frac{1}{\mu_{\text{eff}}} = \frac{1}{\mu_0} + \frac{10^{pK}}{\mu_0} \cdot [\text{OH}^-] \qquad (5)$$

From the intercept and slope of the linear regression line, shown in Fig. 5, the mobility at full protonation and the pK_b value were calculated respectively. The pK_a $(14 - pK_b)$ was determined as 10.1, significantly higher than the literature value of 9.1. The μ_0 value was $19.38 \cdot 10^{-9}$ m^2/V s.

In the experiments with non-zero TRIME-β concentrations, the BGE viscosity η was increased. This viscosity was found to be related to the TRIME-β concentration in a non-linear fashion, according to Eq. (1), with $a_1 = 2.9$ and $a_2 = 40$. The mobilities were then corrected using Eq. (2) with $a_3 = 0.78$.

Next, using an ε-aminocaproic acid–acetate buffer of pH 5 and ionic strength 10 mM, the effective mobilities of the optical isomers were determined at different concentration levels of TRIME-β in the range 0–80 mM. Under these conditions, m-fenfluramine was completely ionized, so that only K_2 values were relevant. Results are plotted in Fig. 6. From the slope of the linear regression lines, values of K_2 and δK_2 were determined. The x-axis intercept yielded the mobility of the analyte–selector complex. Table 2 gives a summary of results. Statistical analysis of the mobilities of the analyte–selector complexes has shown that there was no chiral distinction between the two values.

Finally, using 10 mM sodium borate pH 9.46 as BGE, effective mobilites were measured at different TRIME-β concentrations. Under these conditions, both ionic and non-ionic forms of m-fenfluramine were present. The resulting effective mobilities, together with the values for μ_0, K_2, K_b, R_M and $[\text{OH}^-]$ were inserted into Eq. (4), which was then linearized as follows:
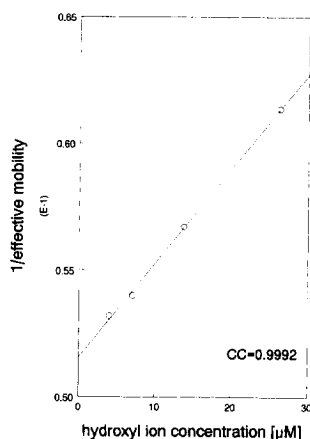

Fig. 5. Experimental determination of pK and μ_0 values of m-fenfluramine at 25°C and 10 mM. The inverse effective mobility (mobility in units of 10^{-9} m^2/V s) is plotted vs. the hydrogen ion concentration (in μmol/l). Further details see Section 3.6.
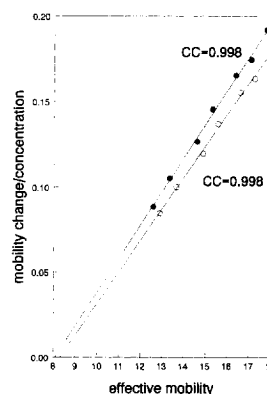

Fig. 6. Experimental determination of K_2 and R_M values of optical isomers of m-fenfluramine at pH 5, using TRIME-β. The $(\mu_0 - \mu_{\text{eff}})/C_s$ is plotted vs. μ_{eff} with mobilities in units of 10^{-9} m^2/V s, C_s in mol/l.

Table 2
Experimental values of electrophoretic and chiral parameters of m-fenfluramine (Conditions as in Section 2)

Parameter	Value	Standard deviation
μ_0	$19.38 \cdot 10^{-9}$ m^2/V s	$0.1 \cdot 10^{-9}$ m^2/V s
pK_a	10.1	0.05
$\mu_{complex}$	$8.2 \cdot 10^{-9}$ m^2/V s	$0.1 \cdot 10^{-9}$ m^2/V s
R_M	41%	0.5%
K_1	56	2.1
δK_1	0%	–
K_2	19.2	0.2
δK_2	6.3%	0.1%

$$(1 + R_M K_2 C_s)\mu_0/\mu_{eff} - 1 - K_2 C_s - [OH^-]/K_b$$
$$= K_1 C_s [OH^-]/K_b \qquad (6)$$

Fig. 7 shows the left-hand side of Eq. (6) plotted vs. C_s in mM units, so that the slope of the line equals $K_1[OH^-]/K_b$. A linear relation is obvious between 0 and 40 mM TRIME-β, corresponding to K_1 values of 55.4 and 56.6, respectively. Standard deviations were 1.8 and 2.0 respectively, so that it can be safely assumed that there is no chiral discrimination of the non-ionic form of m-fenfluramine ($\delta K_1 = 0$). According to the definition by Wren et al. [15], the K_1 values correspond to an optimum TRIME-β concentration of 5 mM. At much higher concentrations (60–80 mM), an unexplained deviation from the linear relation was observed. The y-axis intercept of the lines in Fig. 7. indicates that the free m-fenfluramine has an effective charge of 0.186 at this pH.
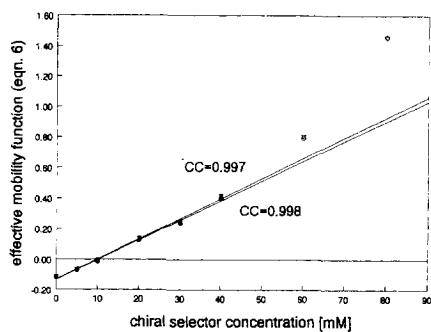
Table 2 summarizes all chiral parameters of m-fenfluramine interacting with TRIME-β.

The separation of a typical Isomeride sample was now simulated, using the chiral parameter values in Table 2 as model input. Experimental conditions were 30 kV in a 50-μm capillary of length 400 mm (470 mm to the detector). The buffer was a 100 mM β-alanine–acetate buffer with pH 4.2 and 40 mM TRIME-β. The simulation is shown in Fig. 8, the actual separation, under the same conditions in Fig. 9. The comparison is satisfactory.

4. Conclusions

The above mentioned chiral parameters, together with the original features of the program, provide a very flexible training tool for chiral separations in

Fig. 7. The left hand side of Eq. (6) including the effective mobility (in units of 10^{-9} m^2/V s) of m-fenfluramine enantiomers at pH 9.5 vs. the concentration of TRIME-β.
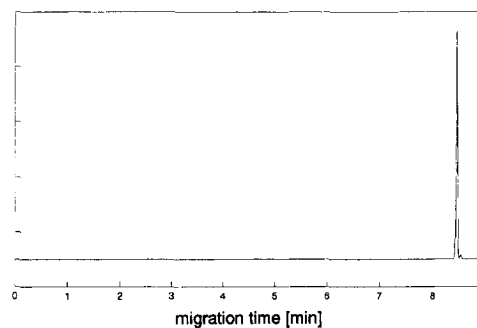

Fig. 8. Simulation of the chiral separation of a sample of Isomeride with 97% purity. Chiral parameters as in Table 2, other conditions as mentioned in Section 3.6.
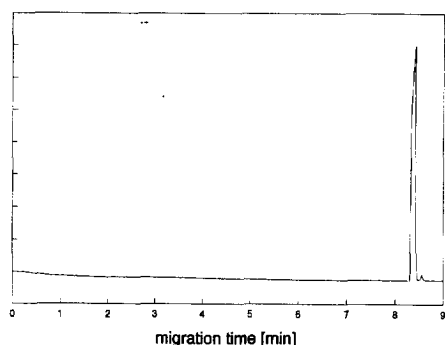
Fig. 9. Experimental determination of Isomeride with 97% optical purity, conditions identical to those of Fig. 8.

capillary electrophoresis. For example, determination of the optimum chiral selector concentration can be done in seconds, using the simulation software. Although the software is intended mainly for training purposes, in cases where sufficient details of existing chiral selector–analyte combinations are available, the program is also suitable for method development of chiral separations in CE.

Acknowledgments

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